enabling comparison of the amount of said extracellular mRNA in an individual's bodily fluid with the range of amounts of said mRNA present in the bodily fluids of populations with cancer, premalignancy, or normal populations without cancer.

The methods of the invention as described above is not limited to blood plasma or serum, and can be performed in like manner for detecting extracellular EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 RNA or any combination thereof from other bodily fluids, including but not limited to whole blood, urine, effusions, ascitic fluid, saliva, cerebrospinal fluid, cervical secretions, vaginal secretions, endometrial secretions, amniotic fluid, gastrointestinal secretions, breast fluid or secretions, and bronchial secretions including sputum. Whereas fractionation of the bodily fluid into its cellular and non-cellular components is not required for the practice of the invention, the non-cellular fraction may be separated, for example, by centrifugation or filtration of the bodily fluid.

The methods of the invention are useful in the practice of diagnostic methods for detecting extracellular mRNA in an animal, most preferably a human at risk for developing or who has developed a premalignant or malignant neoplastic disease comprising cells expressing EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 RNA or any combination thereof. The invention further provides a method of identifying animals, particularly humans at risk for developing, or who have developed premalignancies or cancer of epithelial tissues and components of tissues, including but not limited to breast, ovarian, lung, cervical, colorectal, gastric, pancreatic, bladder, prostate, head and neck, endometrial, kidney, and esophageal cancers, as well as premalignancies and carcinoma in-situ including but not limited to cervical dysplasia and cervical intraepithelial neoplasia (CIN), bronchial dysplasia, atypical hyperplasia

of the breast, ductal carcinoma in-situ, colorectal adenoma, atypical endometrial hyperplasia, and Barrett's esophagus.

The diagnostic methods of the invention can be advantageously performed using a diagnostic kit as provided by the invention, wherein the kit includes oligonucleotide primers specific for cDNA synthesis of EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 RNA or any combination thereof, or *in vitro* amplification or both, or specific probes, most preferably oligonucleotide probes for detecting EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 RNA or associated hnRNP A2 or B1 RNA or any combination thereof or corresponding cDNA or *in vitro* amplified DNA fragments thereof. The kit can further include methods and reagents for extracting extracellular RNA from a bodily fluid, wherein the bodily fluid is most preferably but not limited to blood plasma or serum. The kit can further comprise reagents for reverse-transcribing said RNA into cDNA or reagents for performing *in vitro* amplification. The kit can further comprise instructions for performing methods for extracting RNA from the bodily fluid, reverse-transcribing said RNA into cDNA or for performing *in vitro* amplification.

The inventive methods have significant advantages in assigning and monitoring therapies not specifically directed at cells expressing EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 or any combination thereof, including chemotherapy, radiation therapy, and surgery. The inventive methods further have significant advantages in assigning and monitoring therapies directed at cells expressing EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 or any combination thereof, such as specific or directed therapies such as monoclonal antibody therapies directed at EGFr or her-2/neu, exemplified by Herceptin (Genentech), a her-2/neu-directed monoclonal antibody, and C225 (ImClone Systems), an EGFr-directed monoclonal antibody, or tyrosine kinase inhibitors and small molecule therapies, anti-sense therapies, and vaccine therapies. The methods of the

invention permit stratification and selection of individuals, particularly individual human patients likely to benefit from these specific or directed therapies. The inventive methods are also useful for monitoring response, relapse, and prognosis of neoplastic disease associated with expression of EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 or any combination thereof. Of particular value, the invention allows a determination that a directed therapy is therapeutically indicated even in cases of premalignancy, early cancer, or occult cancers or minimum residual disease, as well as when metastatic disease is present. Thus, the invention permits therapeutic intervention when tumor burden is low, immunologic function is relatively intact, and the patient is not compromised, all increasing the potential for cure.

The methods of the invention further enable RNA encoding EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1, or any combination thereof, to be evaluated in blood plasma, serum or other bodily fluid in combination with detection of other tumor-associated or tumor-derived RNA or DNA in a concurrent or sequential fashion, such as in a multiplexed assay or in a chip-based assay, thereby increasing the sensitivity or efficacy of the assay in the detection or monitoring of neoplastic disease. For example, EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 RNA, or any combination thereof can be detected in blood, plasma, serum, or other bodily fluid in combination with detection of telomerase-associated RNA such as hTR and/or hTERT, or in combination with detection of cancer-associated viral DNA such as human papillomavirus DNA, or in combination with oncogene DNA such as mutated K-ras DNA, or in combination with tumor suppressor gene DNA such as altered P53 or APC DNA and/or in combination with microsatellite DNA.

The methods of the invention and preferred uses for the methods of the invention are more fully illustrated in the following Examples. These Examples illustrate certain aspects of